

Amendments to the Claims:

Please amend the claims as noted below, without prejudice to subsequent renewal. The listing of claims below replaced all prior versions, and listings, of claims in the application.

1. (Withdrawn) A four helical bundle (4HB) polypeptide comprising one or more non-naturally encoded amino acids.
2. (Withdrawn) The 4HB polypeptide of claim 1, wherein the 4HB polypeptide comprises one or more post-translational modifications.
3. (Withdrawn) The 4HB polypeptide of claim 1, wherein the polypeptide is linked to a linker, polymer, or biologically active molecule.
4. (Withdrawn) The 4HB polypeptide of claim 3, wherein the polypeptide is linked to a water soluble polymer.
5. (Withdrawn) The 4HB polypeptide of claim 1, wherein the polypeptide is linked to a bifunctional polymer, bifunctional linker, or at least one additional 4HB polypeptide.
6. (Withdrawn) The 4HB polypeptide of claim 5, wherein the bifunctional linker or bifunctional polymer is linked to a second polypeptide.
7. (Withdrawn) The 4HB polypeptide of claim 6, wherein the second polypeptide is a 4HB polypeptide.
8. (Withdrawn) The 4HB polypeptide of claim 4, wherein the water soluble polymer comprises a poly(ethylene glycol) moiety.
9. (Withdrawn) The 4HB polypeptide of claim 4, wherein said water soluble polymer is linked to a non-naturally encoded amino acid present in said 4HB polypeptide.
10. (Withdrawn) The 4HB polypeptide of claim 1, selected from the group consisting of G-CSF, erythropoietin, interferon, and growth hormone.

11. (Withdrawn) The 4HB polypeptide of claim 1, wherein the 4HB polypeptide comprises one or more amino acid substitution, addition or deletion that modulates affinity of the 4HB polypeptide for a 4HB receptor.

12. (Withdrawn) The 4HB polypeptide of claim 1, wherein the 4HB polypeptide comprises one or more amino acid substitution, addition or deletion that increases the stability or solubility of the 4HB polypeptide.

13. (Withdrawn) The 4HB polypeptide of claim 1, wherein the 4HB polypeptide comprises one or more amino acid substitution, addition or deletion that increases the expression of the 4HB polypeptide in a recombinant host cell or synthesized in vitro.

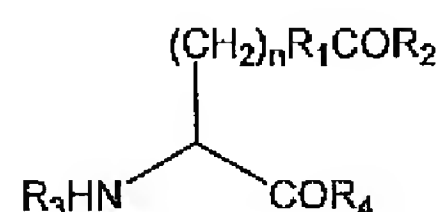
14. (Withdrawn) The 4HB polypeptide of claim 1, wherein the 4HB polypeptide comprises one or more amino acid substitution, addition or deletion that increases protease resistance of the 4HB polypeptide.

15. (Withdrawn) The 4HB polypeptide of claim 1, wherein the non-naturally encoded amino acid is reactive toward a linker, polymer, or biologically active molecule that is otherwise unreactive toward any of the 20 common amino acids in the polypeptide.

16. (Withdrawn) The 4HB polypeptide of claim 1, wherein the non-naturally encoded amino acid comprises a carbonyl group, an aminooxy group, a hydrazine group, a hydrazide group, a semicarbazide group, an azide group, or an alkyne group.

17. (Withdrawn) The 4HB polypeptide of claim 16, wherein the non-naturally encoded amino acid comprises a carbonyl group.

18. (Withdrawn) The 4HB polypeptide of claim 17, wherein the non-naturally encoded amino acid has the structure:



wherein n is 0-10; R1 is an alkyl, aryl, substituted alkyl, or substituted aryl; R2 is H, an alkyl, aryl, substituted alkyl, and substituted aryl; and R3 is H, an amino acid, a polypeptide, or an amino terminus modification group, and R4 is H, an amino acid, a polypeptide, or a carboxy terminus modification group.

19. (Withdrawn) The 4HB polypeptide of claim 16, wherein the non-naturally encoded amino acid comprises an aminooxy group.

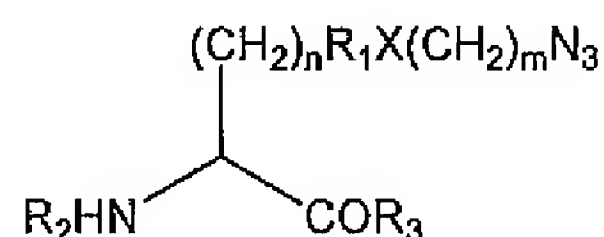
20. (Withdrawn) The 4HB polypeptide of claim 16, wherein the non-naturally encoded amino acid comprises a hydrazide group.

21. (Withdrawn) The 4HB polypeptide of claim 16, wherein the non-naturally encoded amino acid comprises a hydrazine group.

22. (Withdrawn) The 4HB polypeptide of claim 16, wherein the non-naturally encoded amino acid residue comprises a semicarbazide group.

23. (Withdrawn) The 4HB polypeptide of claim 16, wherein the non-naturally encoded amino acid residue comprises an azide group.

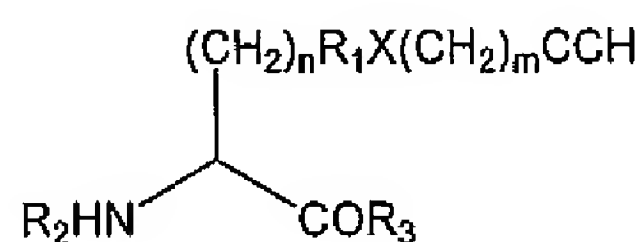
24. (Withdrawn) The 4HB polypeptide of claim 23, wherein the non-naturally encoded amino acid has the structure:



wherein n is 0-10; R1 is an alkyl, aryl, substituted alkyl, substituted aryl or not present; X is O, N, S or not present; m is 0-10; R2 is H, an amino acid, a polypeptide, or an amino terminus modification group, and R3 is H, an amino acid, a polypeptide, or a carboxy terminus modification group.

25. (Withdrawn) The 4HB polypeptide of claim 16, wherein the non-naturally encoded amino acid comprises an alkyne group.

26. (Withdrawn) The 4HB polypeptide of claim 25, wherein the non-naturally encoded amino acid has the structure:



wherein n is 0-10; R1 is an alkyl, aryl, substituted alkyl, or substituted aryl; X is O, N, S or not present; m is 0-10, R2 is H, an amino acid, a polypeptide, or an amino terminus modification group, and R3 is H, an amino acid, a polypeptide, or a carboxy terminus modification group.

27. (Withdrawn) The 4HB polypeptide of claim 4, wherein the water soluble polymer has a molecular weight of between about 0.1 kDa and about 100 kDa.

28. (Withdrawn) The 4HB polypeptide of claim 27, wherein the water soluble polymer has a molecular weight of between about 0.1 kDa and about 50 kDa.

29. (Withdrawn) The 4HB polypeptide of claim 4, which is made by reacting a 4HB polypeptide comprising a carbonyl-containing amino acid with a water soluble polymer comprising an aminooxy, hydrazine, hydrazide or semicarbazide group.

30. (Withdrawn) The 4HB polypeptide of claim 29, wherein the aminooxy, hydrazine, hydrazide or semicarbazide group is linked to the water soluble polymer through an amide linkage.

31. (Withdrawn) The 4HB polypeptide of claim 4, which is made by reacting a water soluble polymer comprising a carbonyl group with a polypeptide comprising a non-naturally encoded amino acid that comprises an aminooxy, a hydrazine, a hydrazide or a semicarbazide group.

32. (Withdrawn) The 4HB polypeptide of claim 4, which is made by reacting a 4HB polypeptide comprising an alkyne-containing amino acid with a water soluble polymer comprising an azide moiety.

33. (Withdrawn) The 4HB polypeptide of claim 4, which is made by reacting a 4HB polypeptide comprising an azide-containing amino acid with a water soluble polymer comprising an alkyne moiety.

34. (Withdrawn) The 4HB polypeptide of claim 16, wherein the azide or alkyne group is linked to a water soluble polymer through an amide linkage.

35. (Withdrawn) The 4HB polypeptide of claim 4, wherein the water soluble polymer is a branched or multiarmed polymer.

36. (Withdrawn) The 4HB polypeptide of claim 35, wherein each branch of the branched polymer has a molecular weight of between about 1 kDa and about 100 kDa.

37. (Withdrawn) The 4HB polypeptide of claim 1, wherein the polypeptide is a 4HB antagonist.

38. (Withdrawn) The 4HB polypeptide of claim 37, wherein the polypeptide comprises one or more post-translational modification, linker, polymer, or biologically active molecule.

39. (Withdrawn) The 4HB polypeptide of claim 38, wherein the polymer comprises a moiety selected from a group consisting of a water soluble polymer and poly(ethylene glycol).

40. (Withdrawn) The 4HB polypeptide according to claim 37, wherein the non-naturally encoded amino acid is present within the Site II region of the 4HB polypeptide.

41. (Withdrawn) The 4HB polypeptide according to claim 37, wherein the polypeptide prevents dimerization of a 4HB receptor.

42. (Withdrawn) The 4HB polypeptide of claim 1, wherein the non-naturally encoded amino acid comprises a saccharide moiety.

43. (Withdrawn) The 4HB polypeptide of claim 3, wherein the linker, polymer, or biologically active molecule is linked to the polypeptide via a saccharide moiety.

44. (Currently Amended) An isolated nucleic acid comprising at least one selector codon that is recognized by an orthogonal translation system and wherein the polynucleotide is translated by the translation system to produce a four helical bundle (4HB) polypeptide agonist selected from the group consisting of human growth hormone, interferon, erythropoietin, and granulocyte cell stimulating factor, and wherein said orthogonal translation system translates the selector codon for a non-naturally encoded amino acid, **and wherein the 4HB polypeptide agonist has increased agonist activity as compared to a wild type 4HB polypeptide of the same type.**

45. (Previously presented) The isolated nucleic acid of claim 44, wherein the selector codon is selected from the group consisting of an amber codon, ochre codon, opal codon, a unique codon, a rare codon, and a four-base codon.

46. (Withdrawn) A method of making the 4HB polypeptide of claim 3, the method comprising contacting an isolated 4HB polypeptide comprising a non-naturally encoded amino acid with a linker, polymer, or biologically active molecule comprising a moiety that reacts with the non-naturally encoded amino acid.

47. (Withdrawn) The method of claim 46, wherein the polymer comprises a moiety selected from a group consisting of a water soluble polymer and a poly(ethylene glycol).

48. (Withdrawn) The method of claim 46, wherein the non-naturally encoded amino acid comprises a carbonyl group, an aminooxy group, a hydrazide group, a hydrazine group, a semicarbazide group, an azide group, or an alkyne group.

49. (Withdrawn) The method of claim 46, wherein the non-naturally encoded amino acid comprises a carbonyl moiety and the linker, polymer, or biologically active molecule comprises an aminooxy, a hydrazine, a hydrazide or a semicarbazide moiety.

50. (Withdrawn) The method of claim 49, wherein the aminooxy, hydrazine, hydrazide or semicarbazide moiety is linked to the linker, polymer, or biologically active molecule through an amide linkage.

51. (Withdrawn) The method of claim 46, wherein the non-naturally encoded amino acid residue comprises an alkyne moiety and the linker, polymer, or biologically active molecule comprises an azide moiety.

52. (Withdrawn) The method of claim 46, wherein the non-naturally encoded amino acid residue comprises an azide moiety and the linker, polymer, or biologically active molecule comprises an alkyne moiety.

53. (Withdrawn) The method of claim 48, wherein the azide or alkyne moiety is linked to a linker, polymer, or biologically active molecule through an amide linkage.

54. (Withdrawn) The method of claim 47, wherein the poly(ethylene glycol) moiety has an average molecular weight of between about 0.1 kDa and about 100 kDa.

55. (Withdrawn) The method of claim 47, wherein the poly(ethylene glycol) moiety is a branched or multiarmed polymer.

56. (Withdrawn) A composition comprising the 4HB polypeptide of claim 1 and a pharmaceutically acceptable carrier.

57. (Withdrawn) The composition of claim 56, wherein the non-naturally encoded amino acid is linked to a water soluble polymer.

58. (Withdrawn) A method of treating a patient having a disorder modulated by 4HB comprising administering to the patient a therapeutically-effective amount of the composition of claim 56.

59. (Previously Presented) An isolated cell comprising the nucleic acid of claim 44.

60. (Previously Presented) The isolated cell of claim 59, wherein the cell comprises an orthogonal tRNA synthetase or an orthogonal tRNA.

61. (Cancelled).

62. (Withdrawn) A method of increasing serum half-life or circulation time of a 4HB polypeptide, the method comprising substituting one or more non-naturally encoded amino acids for any one or more naturally occurring amino acids in the 4HB polypeptide.

63. (Withdrawn) A 4HB polypeptide encoded by a polynucleotide, wherein said polynucleotide comprises a selector codon, and wherein said polypeptide comprises at least one non-naturally encoded amino acid.

64. (Withdrawn) The 4HB polypeptide of claim 63, wherein the non-naturally encoded amino acid is linked to a linker, polymer, water soluble polymer, or biologically active molecule.

65. (Withdrawn) The 4HB polypeptide of claim 64, wherein the water soluble polymer comprises a poly(ethylene glycol) moiety.

66. (Withdrawn) The 4HB polypeptide of claim 63, wherein the non-naturally encoded amino acid comprises a carbonyl group, an aminooxy group, a hydrazide group, a hydrazine group, a semicarbazide group, an azide group, or an alkyne group.

67. (Withdrawn) The 4HB polypeptide of claim 65, wherein the poly(ethylene glycol) moiety has a molecular weight of between about 0.1 kDa and about 100 kDa.

68. (Withdrawn) The 4HB polypeptide of claim 65, wherein the poly(ethylene glycol) moiety is a branched or multiarmed polymer.

69. (Withdrawn) The 4HB polypeptide of claim 68, wherein the poly(ethylene glycol) moiety has a molecular weight of between about 1 kDa and about 100 kDa.

70. (Withdrawn) A composition comprising the 4HB polypeptide of claim 63 and a pharmaceutically acceptable carrier.

71. (Withdrawn) A 4HB polypeptide comprising one or more amino acid substitution, addition or deletion that increases the expression of the 4HB polypeptide in a recombinant host cell.

72. (Withdrawn) A 4HB polypeptide comprising a water soluble polymer linked by a covalent bond to the 4HB polypeptide at a single amino acid.

73. (Withdrawn) The 4HB polypeptide of claim 72, wherein the water soluble polymer comprises a poly(ethylene glycol) moiety.

74. (Withdrawn) The 4HB polypeptide of claim 72, wherein the amino acid covalently linked to the water soluble polymer is a non-naturally encoded amino acid.

75. (Withdrawn) The 4HB polypeptide of claim 10 wherein said non-naturally encoded amino acid is linked to a poly(ethylene glycol) molecule.

76. (Withdrawn) A polypeptide comprising at least one linker, polymer, or biologically active molecule, wherein said linker, polymer, or biologically active molecule is attached to the polypeptide through a functional group of a non-naturally encoded amino acid ribosomally incorporated into the polypeptide.

77. (Withdrawn) The polypeptide of claim 76, wherein said polypeptide is monoPEGylated.

78. (Withdrawn) The polypeptide of claim 76, wherein said polypeptide is a 4HB polypeptide.

79. (Withdrawn) A polypeptide comprising a linker, polymer, or biologically active molecule that is attached to one or more non-naturally encoded amino acid wherein said non-naturally encoded amino acid is ribosomally incorporated into the polypeptide at pre-selected sites.

80. (Withdrawn) The polypeptide of claim 79, wherein said polypeptide is a 4HB polypeptide.

81. (Withdrawn) The 4 HB polypeptide of claim 1, wherein the 4HB polypeptide comprises one or more amino acid substitution, addition, or deletion that modulates immunogenicity of the 4HB polypeptide.

82. (Withdrawn) The 4HB polypeptide of claim 1, wherein the 4HB polypeptide comprises one or more amino acid substitution, addition, or deletion that modulates serum half-life or circulation time of the 4HB polypeptide.

83. (Withdrawn) A method of modulating immunogenicity of a 4HB polypeptide, the method comprising substituting one or more non-naturally encoded amino acids for any one or more naturally occurring amino acids in the 4HB polypeptide.